Article type: *Original research paper*

Influence of ground predators on the survival of the Mediterranean fruit fly pupae, *Ceratitis capitata*, in Spanish citrus orchards

Alberto URBANEJA[1], Ferran GARCÍA MARÍ[2], David TORTOSA[1], Cristina NAVARRO[2], Pilar VANACLOCHA[1], Laura BARGUES[2], and Pedro CASTAÑERA[1]

[1]Unitat d'Entomologia IVIA-CIB CSIC. Institut Valencià d'Investigacions Agràries (IVIA). Ctra. de Montcada a Nàquera km. 5; 46113-Montcada (Spain).

[2]Grup d´Entomologia Agroforestal. Institut Agroforestal Mediterrani. Universitat Politècnica de València (UPV). Camí de Vera, 14. 46022 -València (Spain)

Address for correspondence:

Alberto Urbaneja García
Unitat d'Entomologia IVIA-CIB CSIC
Dep. Protecció Vegetal i Biotecnologia
Institut Valencià d'Investigacions Agràries (IVIA).
Ctra. Montcada –Nàquera Km. 4,5
46113 Montcada, València (SP)
Tel: +34 963424000
Fax: +34 963424001
E-mail: aurbaneja@ivia.es

Abstract

A survey of predaceous ground arthropods was conducted in two citrus orchards in València, Spain, and the roles of these as predators of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) pupae was evaluated under field and laboratory conditions. A total of 2,950 predaceous arthropods were collected in the two orchards from July 2003 until September 2004. Ants (Hymenoptera) were the most abundant group (83%), followed by Aranae (≈5%), Staphylinidae (≈8%), Carabidae (<1%), Cicindelidae (<1%) and Dermaptera (≈1%). Pupae disappearance rates were higher in the period of the year (warmer months of the year, from May to October) and in the orchard (plot 2), where ant populations were greatest. Average survival of *C. capitata* pupae in the warm season was 35.7 ± 6.2% and 14.3 ± 6.7% in both orchards. Symptoms of predation, inferred from broken or abnormal pupae, were more frequently observed in the colder months, from November to April, when spiders, Staphylinidae and other predators were present. The combined effect of predation and low temperature during the one to four months the pupae stayed in the soil during the cold season resulted in only 10.0 ± 6.0% and 4.7 ± 3.4% of adult emergence in both orchards. In no-choice laboratory trials, all the predator species tested were able to feed on the *C. capitata* pupae, with statistical differences in feeding rates between species. Preliminary data show that Carabids include the most voracious species, consuming more than one pupa per day followed in order of importance by Cicindelidae, earwigs and spiders.

Key Words: Diptera, Tephritidae, Carabidae, Staphylinidae, Cicindelidae, Formicidae, Dermaptera, Aranae, Medfly, citrus.

Introduction

The Mediterranean fruit fly or Medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most devastating fruit pests worldwide because of its global distribution (EPPO, 2004), wide range of hosts (Liquido *et al*., 1991), rapid dispersion (Papadopoulos *et al.*, 1996 and 2003) and adaptation to low temperatures (Del Pino, 2000; Putruelle, 1998). Current control of Medfly in Spain is mainly based on field monitoring and both aerial and terrestrial treatments with organophosphate insecticides, especially malathion, mixed with protein baits (Primo *et al.*, 2003). In recent years, emphasis has been placed on implementing environmentally safe measures to control *C. capitata* in Spain, rather than using traditional chemical insecticides (Castañera, 2003).To this end, two different lines of research in biological control are currently being followed. For the first, a classical biological control program was initiated in 2002 by introducing two exotic parasitoids from Hawaii, *Fopius arisanus* (Sonan) and *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae) (Falcó *et al*., 2003). For the second, which includes this study, the role of polyphagous predators on *C. capitata* are being evaluated under Mediterranean conditions. The final goal of these research programs is to develop tools and conservation strategies that will enable an IPM system to be implemented in citrus crops and, accordingly, to optimize Medfly management both economically and environmentally.

In general, the quantitative effects of predators on *C. capitata* mortality have been studied less than those of parasitoids (Debouzie, 1989). However, pupal mortality may be important in regulating the abundance of fruit fly populations in the field (Bateman, 1976; Hogdson *et al*, 1998; Wong *et al*, 1984). This mortality is usually attributed to predation by several arthropods. Among them, ants, carabid and staphylinid beetles, and spiders are often cited as preying on larvae and pupae of fruit flies (Allen, 1990; Eskafi and Kolbe 1990; Galli and Rampazzo, 1996).

Hitherto, a survey of Medfly predators has not been carried out in the Spanish citrus ecosystem. We report here on the abundance of the soil predators in two citrus orchards in València, Spain as well as on their potential role on the survival of *C. capitata* pupae under field and laboratory conditions.

Materials and Methods

Sampling sites

Two citrus orchards of 0.4 ha located in Picassent [Spain (39º, 20.862' N; 0º, 30.095' W; 41 m altitude)] were used. Both orchards, surrounded by other citrus orchards, were drip-irrigated on bare soil, and pests and weeds were controlled using conventional farm practices. One of the orchards (Plot 1) received during the sampling period four pesticide applications (mineral oil, pyriproxifen, carbosulfan and malathion), whereas the other one (Plot 2) received three (mineral oil, chlorpyriphos and pyriproxifen). The herbicide glyphosate was applied in spring and a shredder was used once for weed control during the fall. Mean air temperatures in Picassent throughout the field sampling period are showed in Figure 1 (INM, 2004).

Ground predators

The activity of ground predators was studied with pitfall traps. Four pitfall traps were regularly distributed per orchard. They consisted of a plastic jar (12.5 cm diameter and 12 cm depth), with a plastic funnel fitted to its upper edge. A plastic container of 150 cc half filled with a 3:1 mixture of water and ethanol, and 0.1% detergent, was placed inside the plastic cup. Sampling was performed from August 2003 until September 2004. Traps were changed every 15 days. All the individuals collected were identified up to genus/species level under a stereomicroscope.

Mortality of Medfly pupae in the field

The mortality of pupae under field conditions was studied from July 2003 to July 2004. Ten popping larvae of *C. capitata*, taken from a reference laboratory colony, were buried in the soil under the tree canopy at an approximate depth of 5 cm in a circular area of 10 cm in diameter. Pupae were covered with soil and gently pressed, and each group of pupae was marked using a toothpick.

Pupae mortality was assessed before and after adult emergence. The observation before adult emergence provides an estimate of the intensity of the mortality factors acting on the Medfly pupae buried in the soil, i.e. mortality per unit of time. The observation after adult emergence allows an estimate of total mortality affecting the Medfly pupae during their stay in the ground.

To assess mortality before adult emergence, pupae were recovered from the soil one week later during the warmer months of the year (May to October), when the estimated period of pupal development ranged between one to two weeks because temperature was usually above 15ºC (Figure 1), or two weeks during the colder months of the year (November to April) when the pupal development was longer than two weeks, with temperatures below 15ºC. A core of soil of 15 cm depth by 15 cm in diameter was removed in and around the place where pupae were buried, and carried to the laboratory. This portion of soil was enough to guarantee the recovery of all pupae (Del Pino, 2000). Soil was carefully inspected under a stereomicroscope to separate the pupae in three categories: healthy, dead or hatched pupae. Dead pupae were separated into groups according to their appearance and ascribed to specific predators according to laboratory trials (see below). Pupae with a normal appearance were allowed to emerge in a climatic chamber, at 25 ± 3ºC, 60-80% RH and a 16:8 L:D photoperiod.

To assess mortality after adult emergence, one week (warmer months) or two weeks (colder months) after burying the popping larvae, that site was covered with an emergence cage consisting of a polyethylene cylinder (10 cm diameter by 15 cm high) top-screened with a 60-mesh nylon screen. A yellow sticky trap (3x3 cm) coated with Tanglefoot® (Tanglefoot Company, Grand Rapids, Michigan, USA) was hung inside the cylinder to collect emerging Medfly adults. During the warmer months these cylinders were left undisturbed in the field for another week. This gave time enough for all the pupae to develop into adults. Adults stuck to the trap were counted and soil was excavated and treated as previously described. During the colder months cylinders were left in place indefinitely until all live pupae developed into adults and no further emergence was observed. In this case the soil was not excavated to look for pupal remains because, given the long period of time that had elapsed (one to four months), most had deteriorated.

For every orchard and type of mortality, four replicates were carried out per month, except for July and August 2003 when eight replicates per month were considered.

Predation of Medfly pupae in the laboratory

The capacity of six polyphagous predators to feed on *C. capitata* pupae was evaluated under laboratory conditions by using a no-choice test. Predators were selected from among the most abundant species previously found in the survey carried out in the field. The following species were tested: two Carabidae [*Pseudophonus rufipes* (Duftschmid) (n=11) and *Harpalus distinguendus* (Degeer) (n=8)], one Cicindelidae [*Cicindela* sp. (n=6)], one Staphylinidae [*Ocypus olens* (Müller) (n=3)], two dermaptera [*Forficula auricularia* (L.) (n=3) and *Euborellia moesta* (Gené) (n=11)] and one spider [Lycosidae sp. (n=10)]. Predators were collected directly from fields surrounding the Institut Valencià d'Investigacions Agràries (IVIA) facilities using empty pit fall traps. These traps were placed in the field for 4-5 hours, in order to collect live predators. Predators were taken to the laboratory immediately, where the tests were conducted.

One predator and 10 *C. capitata* pupae (2 days old) from a reference colony were placed in a 1.5 cm depth layer of dampened pearlite (Floreal, Agroperlita F-13®; Semillas Diago S.L. Picassent, València SP) in a petri dish (140 mm in diameter). Petri dishes were sealed with Parafilm® and left undisturbed at 25 ± 4ºC and a photoperiod of 16:8 L:D. Predators were moved to a new petri dish containing 10 pupae every 3 days, until the predator died. Pupae were checked for symptoms of predation under a stereomicroscope and those with a healthy appearance were left undisturbed in Parafilm®-sealed petri dishes (55 mm in diameter) until adult emergence.

Data analysis

Data from laboratory comparing number of pupae consumed by different predator species and field data comparing the mortality factors between seasons for each plot were subjected to a one-way variance analysis (ANOVA). Field data comparing the factors of mortality of *C. capitata* pupae between the two plots were subjected to a two-way variance analysis (ANOVA), with orchard and sampling date as main factors. The least significant difference (LSD) multiple range test was used for mean separation at $P<0.05$ (SPSS, 1999). Mortality data were transformed using arcsine [square root (p)] for proportion of mortality before each ANOVA was performed. Influence of the ants in the number of disappeared *C. capitata* pupae was studied by plotting the percentage of pupae disappeared against the corresponding mean densities of ants (ants/trap/day), and the correlation equation was calculated using the SPSS software package (SPSS, 1999).

Results

Ground predators

A total of 694 and 2246 predatory arthropods were collected in the plot 1 and 2, respectively. Ants (Hymenoptera) were most abundant, followed by spiders (Aranae), rove beetles (Staphylinidae), ground beetles (Carabidae) and earwigs (Dermaptera). All were more abundant in plot 2 except for Staphylinidae and Carabidae.

Ants represented about 83% of the total numbers of predators collected, with *Messor structor* Bondroit, *Pheidole pallidula* (Nylander) and *Formica fusca* (L) being the three most abundant species (Table 1). Ants were captured in very low numbers during the winter months (end November 2003 until April 2004) (Figure 2). However, they were very abundant during the rest of the year. Staphylinidae represented nearly 8% of the predators (Table 2) and were active in the soil throughout the year, fluctuating in abundance without a clear trend (Figure 3). The most abundant species was *Platystethus cornotus* Gravenhorst (≈ 45%) followed in order of importance by *Atheta* (*Xenota*) *mucronata* (Kraatz) and *Anotilus inustus* (Gravenhorst). Spiders, with about 5%, included *Nemesia dubia* Cambridge as the most common species (Table 3). Other common spiders were the Lycosidae *Pardosa cribata* Simon and *Alopecosa accentuata* (Latreille). Spiders were also present throughout the whole year, with an apparent decrease during the winter (Figure 4). Three species of Dermaptera (≈ 1%) were found (Table 4), *Forficula auricularia* L. (the most abundant), *Euborellia moesta* (Gené) and *Euborellia annulipes* (Lucas). Only two species of Carabidae (0.67%) were identified, the most abundant being *Pseudophonus rufipes* (Degeer) (Table 2). Both carabids and earwigs were captured throughout the whole year. The predator *Cicindela* sp. (Cicindelidae) was also collected in low numbers (0.61%) but only in the spring period (Table 2).

Mortality of Medfly pupae in the field

Differences in mortality factors were considerable when comparing different months of the year (Figure 5). In both plots, disappearance of pupae, was observed throughout the sampling period, but tended to be greater during the warmer months of the year, from May to October. In contrast, other types of predation, inferred from broken or abnormal pupae, were higher during the colder months, from November to April. Mortality due to unknown causes appeared almost exclusively between November and April.

There were considerable overall differences between orchards in the survival of *C. capitata* pupae (F = 10.05; df = 1, 12; P = 0.008). On average, 31.6 ± 5.6% of pupae remained alive after the one or two-week period in the soil in the plot 1, compared with 13.8 ± 4.8% in the plot 2 (Table 5). Differences between the two orchards were found mostly in the disappearance rate of pupae, which was higher in the plot 2 (67.6 ± 6.4%) than in the plot 1 orchard (46.5 ± 5.7%) (F = 11.37; df = 1, 12; P = 0.005). Furthermore, pupae with symptoms of predation were also observed in both plots, although in a smaller percentage (≈ 7%). The predation symptoms, which ranged from empty and broken pupae to pupal fragments, were confirmed after observation in laboratory tests. A low percentage of predated pupae, with characteristic round holes in the pupal tegument, were unequivocally attributed to spiders. Finally, dead pupae showing no symptoms of predation were included in the group of unknown causes of mortality.

In the warmer period (from July throughout October 2003), development of pupae in the soil lasted from 8 to 12 days. When comparing the percentage of mortality one week after burial (before adult emergence) or two weeks (after adult emergence), both methods yielded very similar results, with no statistical differences ($F = 0.42$; $df = 1, 6$; $P = 0.54$ and $F = 0.87$; $df = 1, 6$; $P = 0.39$, for the plots 1 and 2, respectively). Data on mortality measured before and after adult emergence were consequently pooled together to estimate the average total mortality throughout this period. The average survival of the Medfly pupae during their residency in the soil in the warm season was 35.7 ± 6.2% and 14.3 ± 6.7% in the plots 1 and 2, respectively.

In contrast, survivorship and mortality rates were estimated during the cold season (November to April) two-weeks after burying the pupae, well before adult emergence (one to four months later). Thus, mortality during the first week (Figure 5 and Table 5) approximated total mortality during the summer but gave only a time relative estimate of the mortality that occurred during the colder months. The current survival of pupae in the cold season, measured in pupae left until adults emerged, averaged 10.0 ± 6.0% in the plot 1 and 4.7 ± 3.4% in the plot 2.

Predation of Medfly pupae in the laboratory

All the predator species tested in a no-choice test were able to feed on the Medfly pupae (Table 6). The total number of pupae offered was 3,726, of which 1,191 were predated (32%). The number of Medfly pupae predated per day was statistically different between the species (F = 4.6; *df.* = 4, 45; P = 0.004). Carabids, with more than 1 pupae predated per day, and *Cicindela* sp., with 0.6, were found to be the most voracious predators [except for *O. olens* (with 1.6) which was excluded from the analysis because of low numbers tested], followed in order of importance by earwigs and spiders.

Discussion

Although the reports on surveys of the arthropod predator fauna from the canopy of the citrus trees are abundant and its fauna is well-known (Alvis, 2003; Ripollés *et al*., 1995), little information about the predaceous soil arthropods in the citrus ecosystem exists in Spain, apart from that about ants. In this work, species of arthropods belonging to the orders Coleoptera, Dermaptera and Aranae have been determined for the first time in citrus orchards in Spain. Some of the ant species recorded in the current experiment were previously reported in citrus orchards by Palacios *et al*. (1999), who found 14 species in the soil using pitfall traps in Tarragona (250 km north from our study location) and by Alvís (2003), who found 13 species in the canopy of the citrus tree in València.

Ants were the largest group of predators collected in this work. In Guatemala, Eskafi and Kolbe (1990) also found ants to be the most abundant predators in the soil of citrus orchards, followed by Staphylinidae, Carabidae, Histeridae, and Dermaptera. Ants play a dual role in agricultural ecosystems. On the one hand, ants can be detrimental to the biological control of homopterans as they interfere with natural enemies in many crops (Jiggins et al., 1993; Queiroz and Oliveira, 2001; Reimer et al., 1993) and particularly in citrus (Haney et al., 1987; Samways et al., 1998). On the other hand, they can have a beneficial impact by directly consuming pest individuals (DeBach and Rosen, 1991; Hölldobler and Wilson, 1996). In Spanish citrus crops, ants were only known for the indirect damage they cause. Ants feed on honeydew excreted by scales, mealybugs, aphids and whiteflies, and, as part of this relationship, they protect these insects from their natural enemies. In this process ants are able to interfere with, and in many cases to disable, biological control. As a consequence, ant control is considered fundamental to carrying out an IPM program based on biological control in Spain (Palacios *et al.*, 1999; Ripollés *et al*., 1995). However, our results point at a need for clarifying the exact role of ants in Spanish citrus orchards, which deserves further investigation.

Some of the orders and families of predators collected here had previously been identified as Tephritid predators elsewhere (Allen and Hagley, 1990; Galli and Rampazzo, 1996; Mansour et al., 1980). Reported predation rates differ considerably between sites but often reach medium to high levels.

In the current work, high disappearance ratios of pupae of *C. capitata* were obtained. This phenomenon was apparently higher in the warmer months of the year and lower in the colder moths. The same trend could be observed for the ant population. Furthermore, the number of pupae that disappeared was higher in the plot 2, where more ants were also collected than at plot 1. Our results show a weak but a significant correlation between disappearance of pupae and presence of ants (Figure 6), suggesting that ants are in part responsible for searching and carrying off buried Medfly pupae (Hodgson et al., 1998). Pupae disappearance could also be attributed in part to other predators, as we found pupae disappearance in winter, when ants were almost inactive. In a citrus and a coffee orchard in Guatemala, Eskafi et al. (1990) found 7 and 25% mortality in *C. capitata* larvae, respectively, caused by ants. In Hawaii, Pemberton and Willard (1918) observed that ants preyed heavily on larvae and pupae of *C. capitata* in the soil, and Wong *et al.* (1984) estimated that the ant *Linepithema* (*Iridomyrmex) humilis* killed 3% of the mature larvae and 39% of teneral adult flies of *C. capitata* in the soil beneath fruit trees. In four different fruit tree orchards from Mexico Hodgson *et al*. (1998) found that 30 to 85% of *Anastrepha* spp. pupae disappeared, presumably due to ants, staphylinids and other predators.

Symptoms of pupal predation were also observed in both orchards. These symptoms were higher in the colder months, when predators, such as rove beetles, earwigs and spiders were present. Accordingly, predation attributed to spiders during these months was higher in the plot 2, where more spiders were also collected than at plot 1. Eskafi *et al.* (1990) found 34 and 47% mortality in *C. capitata* pupae caused by ground predators in a citrus and a coffee orchard, respectively. Based on field and laboratory observations, they assigned this predation mostly to rove beetles encountered in large numbers (eight species) in both orchards and also partly to other predacious coleopterans. In our laboratory trials we could not test the more abundant species of staphylinid found, and only three individuals of *Ocypus olens* were tested.

Abiotic factors, such as moisture content or soil temperature, have also been considered when studying mortality of tephritid pupae in the soil. Thus, Del Pino (2000) found a high mortality rate in *C. capitata* pupae related to temperatures below 12ºC during the winter period. In Malaysia, Sent and Tan (1990) recorded almost 80% of mortality by desiccation or drowning for larvae and pupae of *Bactrocera* (*Dacus*) *dorsalis* (Hendel). In Florida fruit groves, Hennessey (1994) reported up to 33% mortality in *Anastrepha suspensa* (Loew) pupae due to desiccation. The unknown mortality factor of *C. capitata* pupae encountered in the current work was mainly recorded during the winter period. This mortality could be attributed to low temperatures registered from December to March in the study area, with average mean temperatures below 12ºC (Del Pino, 2000; INM, 2004).

Our data show that the survival of Medfly pupae during the winter months is very low and the predatory activity has as much, or even more, influence than temperature, as a mortality factor. In our conditions, we have observed that predators are active throughout the cold season, when they have the opportunity to act on *C. capitata* pupae, since they are available in the ground for up to four months. Indeed, Bateman (1974) showed that the longer pupae were exposed, the higher the mortality rates reached.

In conclusion, we have identified a large number of ground predatory species in citrus groves for the first time, and we have also differentiated predation symptoms on the pupae of *C. capitata* under laboratory and field conditions. Moreover, our findings suggest that pupal mortality is an important factor regulating Medfly populations in citrus orchards in Spain. A substantial part of it could be attributed to predatory activity, specifically to ants and other predators during the warmer months, and to ground beetles, spiders or earwigs during the winter period. Nevertheless, further research is needed to know the role that all these key predators play in the citrus ecosystems.

Acknowledgments

This work was funded by the Instituto Nacional de Investigación y Tecnología Agraria (INIA) (RTA03-103C61), and by the Conselleria d'Agricultura, Pesca i Alimentació de la Generalitat Valenciana. Philip Stansly (SWFREC, Immokalee, University of Florida) and Josep Jacas (Universitat Jaume I, Castelló, Spain) provided useful comments on an early draft of the manuscript. We are grateful to I. Ruíz-Tapiador [Carabidae, (Universidad Politécnica de Madrid, Madrid,)], K. Gómez (Formicidae), F. Pascual [Dermaptera, (Universidad de Granada, Granada)], A. Melic (Aranae, Sociedad Entomológica Aragonesa, Zaragoza SP) and R. Outeruelo [(Staphylinidae, Universidad Complutense de Madrid Madrid)] for the taxonomical confirmation of the species collected, and to T. Pina, O. Dembilio, C. Monzó, S. Santiago and S. Pascual (IVIA) for their time in field sampling and technical assistance.

References

Allen, W.R. and Hagley, E.A. 1990. Epigeal arthropods as predators of mature larvae and pupas of the apple maggot (Diptera: Tephritidae). *Environ. Entomol.* 19: 309-312.

Alonso-Muñoz, A. and Garcia-Marí, F. 2004. Control de *Ceratitis capitata* (Diptera: Tephritidae) en cítricos utilizando trampeo masivo. *Phytoma España* 157: 28-37.

Alvis, L. 2003. Identificación y abundancia de artrópodos depredadores en los cultivos de cítricos Valencianos. Ph.D. Thesis. Dep. Ecosistemas Agroforestales. ETSIA. Universidad Politécnica de Valencia. 189 pp.

Bateman, M.A. 1974. Fruit flies. In: Delucchi, V.L. (Ed.), Studies in Biological Control. IBP 9. Cambridge University Press, Cambridge, pp. 11-49.

Casaña-Giner, V., Gandia-Balaguer, A., Mengod-Puerta, C., Primo-Millo, J. and Primo-Yufera, E. 1999. Insect growth regulators as chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Entomol*. 92: 303-308.

Castañera, P. 2003. Control integrado de la mosca mediterránea de las frutas (*Ceratitis capitata*) en el Comunidad Valenciana. *Phytoma España* 153: 131-133.

DeBach, P. and Rosen, D. 1991. Biological control by natural enemies. Second edition. Cambridge University Press. Cambridge.

Debouzie, D. 1989. Biotic mortality factors in tephritid populations, pp. 221-227. In World Crop Pests, Vol. 3B, Fruit flies. A. S. Robinson and G. Hooper (eds.). New York: Elsevier.

EPPO [European and Mediterranean Plant Protection Organization]. 2004. Distribution Maps of Quarantine Pests for Europe. *Ceratitis capitata*. http://www.eppo.org/QUARANTINE/insects/Ceratitis_capitata/CERTCA_map.htm (27/10/04).

Eskafi, F.M. and Kolbe, M. M. 1990. Predation on larval and pupal *Ceratitis capitata* (Diptera: Tephritidae) by the ant *Solenopsis germinata* (Hymenoptera: Phormicidae) and other predators in Guatemala. *Environ*. *Entomol.* 19: 148-153.

Galli, J.C. and Rampazzo, E.F. 1996. Enemigos naturales predadores de *Anastrepha* (Diptera, Tephritidae) capturados con trampas de gravedad de suelo en huertos de *Psidium guajava* L. *Bol. San. Veg. Plagas* 22: 297-300.

Haney, P.B., Luck, R.F. and Moreno, D.S. 1987. Increases in densities of the citrus red mite, *Panonychus citri* (Acarina: Tetranychidae), in association with the Argentine and, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in Southern California citrus.

Entomophaga 32: 49-57.

Hennessey, M.K. 1994. Depth of pupation of caribbean fruit fly (Diptera: Tephritidae) in soils in the laboratory. *Environ. Entomol*. 23, 1119-1123.

Hodgson, P.J., Sivinski, J., Quintero, G. and Aluja, M. 1998. Depth of pupation and survival of fruit fly (*Anastrepha* spp.: Tephritidae) pupae in a range of agricultural habitats. *Environ. Entomol*. 27: 1310-1314.

Hölldobler, B. and Wilson, E.O. 1996. Viaje a las hormigas. Una historia de exploración científica. Crítica Grijalbo Mondadori. Barcelona. 270pp.

INM, 2004. Valores climatológicos normales. Instituto Nacional de Meteorología. Ministerio de Medio Ambiente. http://www.inm.es/. (26/11/2004)

Jiggins, C., Majerus, M. and Gough, U. 1993. Ant defense of colonies of *Aphis fabae* Scopoli (Hemiptera. Aphididae), against predation by ladybirds. *Br. J. Entomol. Nat. Hist*. 6: 129-138.

Liquido, N.J., Shinoda, L.A. and Cunningham, R.T. 1991. Host plants of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae): an annotated world review. *Misc. Publ. Entomol. Soc. Am*. 77: 1-52.

Mansour, F., Rosen, D. and Shulov, A. 1980. Biology of the spider Chiracanthium mildei (Arachnida : Clubionidae). *Entomophag,* 25: 237-248.

MAPYA. 2004. Orden APA/1657/2004, de 31 de mayo, por la que se establece la norma técnica específica de la identificación de garantía nacional de producción integrada de cítricos. *BOE (Boletín Oficial del Estado)* 137: 20684-20747.

Navarro-Llopis, V., Sanchis-Cabanes J., Ayala I., Casana-Giner, V. and Primo-Yufera, E. 2004. Efficacy of lufenuron as chemosterilant against *Ceratitis capitata* in field trials. *Pest Manag.Sci*. 60: 914-920.

Palacios, R., Martínez-Ferrer, M.T. and Cerdà, X. 1999. Composición, abundancia y fenología de las hormigas (Hymenoptera: Formicidae) en campos de cítricos de Tarragona. *Bol. San. Veg. Plagas* 25: 229-240.

Papadopoulos, N.T., Carey, J.R., Katsoyannos, B.I. and Kouloussis, N.A.1996. Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Ent.. Soc. Amer*. 89: 526-534.

Papadopoulos, N.T., Katsoyannos, B.I. and Nestle, D. 2003. Spatial Autocorrelation Analysis of a *Ceratitis capitata* (Diptera: Tephritidae) Adult Population in a Mixed Deciduous Fruit Orchard in Northern Greece. *Environ. Entomol.* 32: 319-326.

Pemberton, C.E. and Willard, H.F. 1918. A contribution to the biology of fruit-fly parasites in Hawaii. *J. Agric. Res*. 15: 419-465.

Primo, E., Alfaro, F. and Argilés, R. 2003. Plan de actuación contra la mosca de las frutas (*Ceratitis capitata*) en la Comunidad Valenciana. *Phytoma España* 153: 127-130.

Queiroz, J.M. and Oliveira, P.S. 2001. Tending ants protect honeydew-producing whiteflies (Homoptera: Aleyrodidae). *Environ. Entomol*. 30: 295-297.

Reimer, N.J., Cope, M.L. and Yasuda, G. 1993. Interference of *Pheidole megacephala* (Hymenoptera: Formicidae) with biological control of *Coccus viridis* (Homoptera: Coccidae) in coffee. *Environ. Entomol*. 22: 483-488.

Ripollés, J.L., Marsà, M. and Martínez, M.T. 1995. Desarrollo de un programa de control integrado de las plagas de los cítricos en las comarcas del Baix Ebre-Montsià. *Levante Agrícola* 332: 232-248.

Samways, M.J., Grout, T.G. and Prins, A.J. 1998. Ants as citrus pests. In: Bedford, E.C.G., Van Den Berg, M.A, Villiers, E.A. (Eds.), Citrus Pests in The Republic of South Africa. Institute for Tropical and subtropical crops, Nelspruit, pp. 234-242.

Serit, M. and Tan, K.H. 1990. Immature life table of a natural population of *Dacus dorsalis* in a village ecosystem. *Crop Pest Managemen,* 36: 305-309.

Vergoulas, P. and Torné, M. 2003. Spinosad cebo: un sistema innovador para el control de la mosca del mediterráneo. *Phytoma España* 153: 134-138.

Wong, T.T.Y., McInnis, D.O., Nishimoto, J.I., Ota, A.K and Chang, V.C.S. 1984. Predation of the Mediterranean fruit fly (Diptera:Tephritidae) by the argentine ant (Hymenoptera: Formicidae) in Hawaii. *J. Econ. Entomol*. 77: 1454-1458.

FIGURE LEGENDS

Figure 1. Daily ambient air temperature (o) corresponding to the period of the field study in Picassent, València. The monthly average is indicated by a solid line.

Figure 2. Mean number of ants (individuals/trap/day) collected in pitfall traps in two citrus orchards in Picassent (València).

Figure 3. Mean number of staphylind beetles (individuals/trap/day) collected in pitfall traps in two citrus orchards in Picassent (València).

Figure 4. Mean number of spiders (individuals/trap/day) collected in pitfall traps in two citrus orchards in Picassent (València).

Figure 5. Percentage (mean ± SE) survival and mortality factors affecting pupae of *C. capitata* in two citrus orchards, Plot 1 (A) and Plot 2 (B). Values show mortality observed during one week (May to October) or two weeks (November to April). Data from 40 to 160 pupae per month and orchard.

Figure 6. Correlation between the abundance of ants in pitfall traps at each sampling date and the percentage of Medfly pupae disappeared in the same period.

TABLE 1

Number of the ants collected in pitfall traps in two citrus orchards in València, Spain.

	Plot 1	Plot 2	Total
Messor structor Bondroit	5	617	**622**
Pheidole pallidula (Nylander)	97	489	**586**
Formica fusca (L)	116	277	**393**
Messor bouvieri Bondroit	0	279	**279**
Lassius niger (L)	169	53	**222**
Linepithema humile (Mayr)	24	79	**103**
Plagiolepis pygmaea (Latreille)	4	44	**48**
Lasius fuliginosus (Latreille)	7	38	**45**
Hypoponera eduardi (Forel)	40	1	**41**
Camponotus pilicornis (Roger)	2	32	**34**
Lassius sp.	14	7	**21**
Messor barbarus (L)	2	6	**8**
Cardiocondyla elegans Emery	1	1	**2**
Lasius grandis Forel	1	0	**1**
Not identified	22	27	**49**
Total	**504**	**1,950**	**2,454**

TABLE 2

Number of the predatory coleopterans collected in pitfall traps in two citrus orchards in València, Spain.

	Plot 1	Plot 2	Total
Carabidae	**18**	**1**	**19**
Peudophonus (s.tr.) *rufipes* (Degeer)	13	0	**13**
Harpalus distinguendus (Duftschmid)	3	1	**4**
Not identified	2	0	**2**
Staphylinidae	**120**	**110**	**230**
Platystethus cornutus Gravenhorst	38	70	**108**
Atheta (Xenota) mucronata (Kraatz)	50	22	**72**
Anotilus inustus (Gravenhorst)	17	13	**30**
Gauropterus fulgidus (Fabricius)	8	0	**8**
Cordalia obscura (Gravenhorst)	1	1	**2**
Ocypus olens (Müller)	2	0	**2**
Lepidophallus hesperius Erichson	1	1	**2**
Larvae	2	0	**2**
Tasgius (Paratasgius) ater (Gravenhorst)	0	1	**1**
Phloenomus minimus Erichson	1	0	**1**
Not identified	0	2	**2**
Cicindelidae	**17**	**1**	**18**
Cicindela sp.	17	1	**18**
Total	**155**	**112**	**267**

TABLE 3

Number of spiders collected in pitfall traps in two citrus orchards in València, Spain.

	Plot 1	Plot 2	Total
Nemesia dubia Cambridge	4	43	**47**
Pardosa cribata Simon	4	22	**26**
Alopecosa accentuata (Latreille)	1	10	**11**
Dysdera crocota Koch	1	6	**7**
Erigone dentipalpis (Wider)	4	2	**6**
Zodarion pusio Simon	3	1	**4**
Hogna radiata (Latreille)	1	2	**3**
Setaphis sp.	1	2	**3**
Agelenidae sp.	0	3	**3**
Xysticus bliteus (Simon)	0	2	**2**
Glubia dorsalis Latreille	0	2	**2**
Salticidae sp.	1	0	**1**
Not identified	10	30	**40**
Total	**30**	**125**	**155**

TABLE 4

Number of earwigs collected in pitfall traps in two citrus orchards in València, Spain.

	Plot 1	Plot 2	Total
Forficula auricularia L.	4	35	**39**
Euborellia moesta (Gené)	0	13	**13**
Euborellia annulipes (Lucas)	0	4	**4**
Larvae	1	6	**7**
Total	**5**	**58**	**63**

TABLE 5

Percentage of survival (mean[a] ± SE) and mortality factors affecting pupae of *C. capitata* in two citrus orchards in València, Spain. Values show mortality registered during one week (May to October) or two weeks (November to April).

Orchard	Sampling period	Survival (%)	Mortality (%)			
			Disappeared	Predators	Spiders	Unknown
Plot 1	Jul 03-Oct 03	47.5 ± 4.5 a	42.5 ± 2.7ab	5.5 ± 0.6 a	1.25 ± 0.5 a	3.5 ± 1.9 a
	Nov 03-Apr 04	26.7 ± 9.9 a	35.8 ± 11.5 a	7.0 ± 2.5 a	0.5 ± 0.5 a	30.5 ± 9.6 b
	May 04–Jul 04	20.3 ± 3.9 a	72.0 ± 3.1 b	6.7 ± 4.4 a	0	2.0 ± 1.0 a
	Total[a] (Jul 03-Jul 04)	31.6 ± 5.6 B	46.2 ± 6.6 A	6.5 ± 1.4 A	0.6 ± 0.3 A	15.6 ± 5.8 A
Plot 2	Jul 03-Oct 03	12.5 ± 5.9 a	80.0 ± 6.6 b	2.2 ± 0.7 a	0.5 ± 0.3 a	5.2 ± 4.2 a
	Nov 03-Apr 04	13.0 ± 7.5 a	54.8 ± 8.4 a	9.0 ± 1.1 b	4.3 ± 1.1 b	20.3 ± 2.7 b
	May 04–Jul 04	17.0 ± 15.5 a	83.3 ± 15.2 b	0	0	0
	Total[a] (Jul 03-Jul 04)	13.8 ± 4.8 A	69.2 ± 6.4 B	4.8 ± 1.2 A	2.1 ± 0.7 A	11.0 ± 3.1 A

[a] Average mean values of the months of the period, pooling together all the samples collected in each month.

[b] Values in column in each plot followed by a different lowercase letter are significantly different (one-way ANOVA, $P < 0.05$, LSD test). Total means for the two plots followed by a different uppercase letter are significantly different (two-way ANOVA, $P < 0.05$).

TABLE 6

Mean predation of *C. capitata* pupae by different ground predators in a no-choice test.

	N	Nº Pupae offered	Nº Pupae predated	Predated pupae / day
Carabidae				
Pseudophonus rufipes (Degeer)	11	900	491	1.26 ± 0.36 a
Harpalus distinguendus (Duftschmid)	8	826	344	1,13 ± 0,06 a
Cindelidae				
Cicindela campestris L.	6	250	92	0.66 ± 0.16 ab
Staphylinidae				
Ocypus olens (Müller)*	3	310	178	1.66 ± 0.17
Dermaptera				
Forficula auricularia L*	3	230	23	0.31 ± 0.15
Euborellia moesta (Gené)	11	1010	158	0.44 ± 0.13 bc
Aranae				
Lycosidae sp.	10	370	21	0.19 ± 0.10 c

Within each column mean values followed by a different letter are significantly different (P<0,05, LSD test)

*Not included in the statistical analyses, because of the low number of replicates.

FIGURE 1

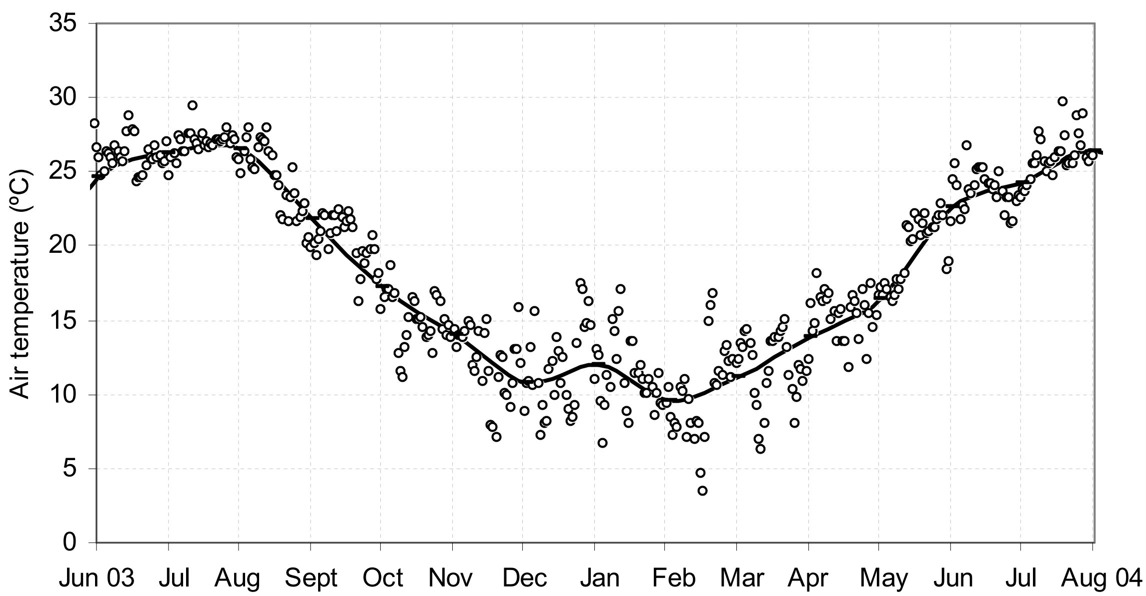

Air temperature (ºC)
35
30
25
20
15
10
5
0
Jun 03
Jul
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug 04

FIGURE 2

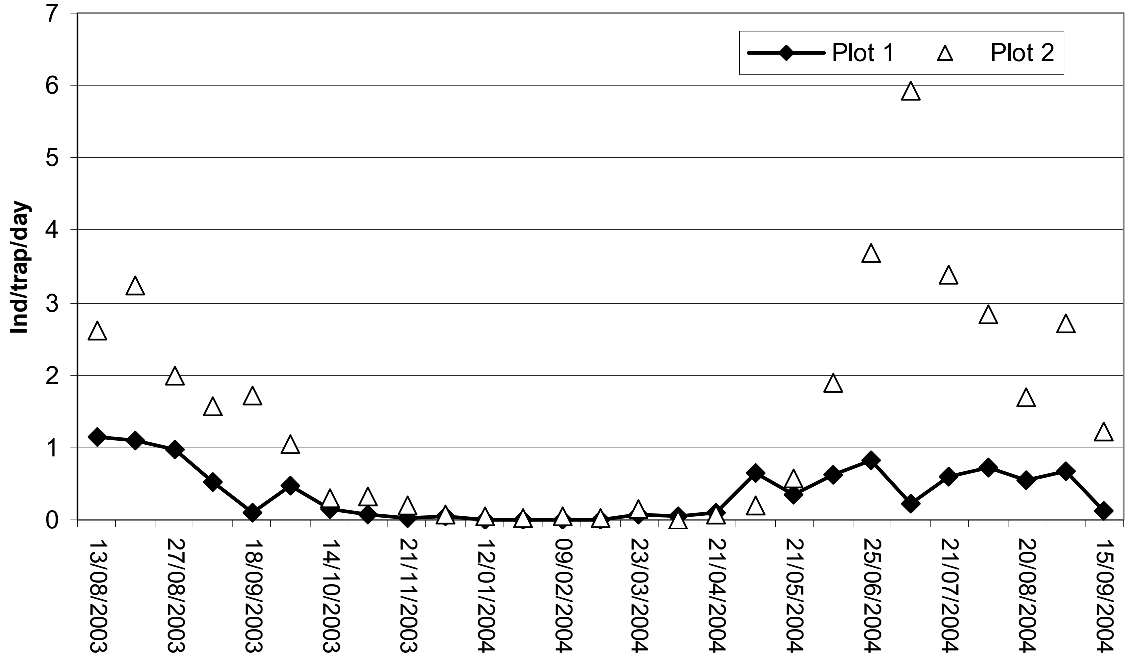

Plot 1
Plot 2
Ind/trap/day
0
1
2
3
4
5
6
7
13/08/2003
27/08/2003
18/09/2003
14/10/2003
21/11/2003
12/01/2004
09/02/2004
23/03/2004
21/04/2004
21/05/2004
25/06/2004
21/07/2004
20/08/2004
15/09/2004

FIGURE 3

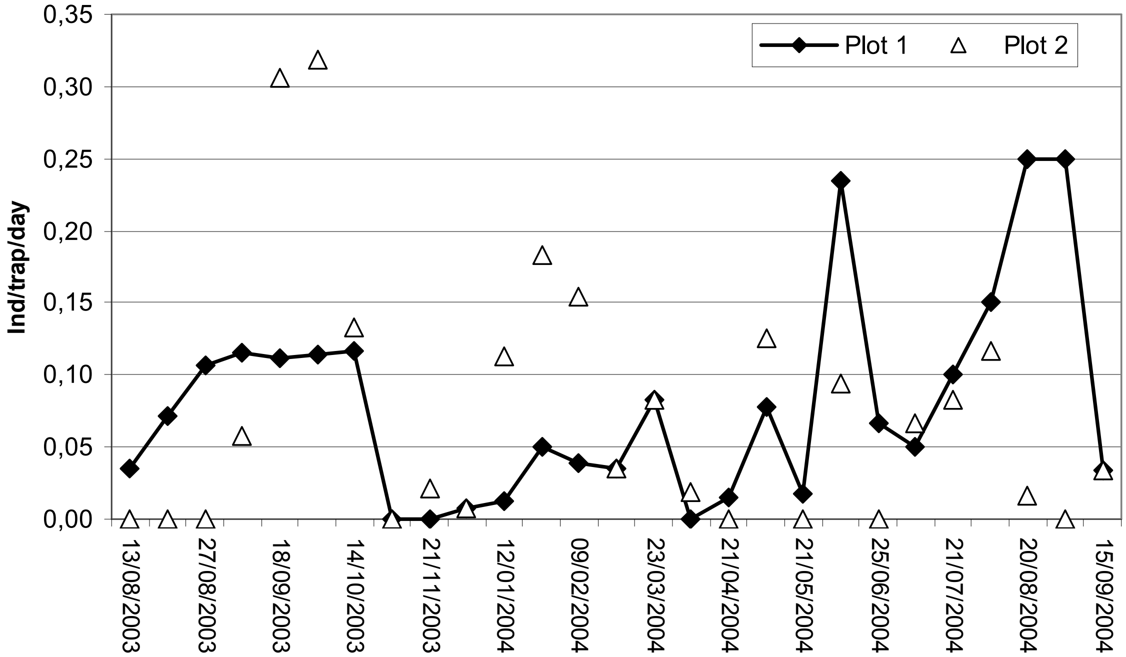

Plot 1
Plot 2
Ind/trap/day
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
13/08/2003
27/08/2003
18/09/2003
14/10/2003
21/11/2003
12/01/2004
09/02/2004
23/03/2004
21/04/2004
21/05/2004
25/06/2004
21/07/2004
20/08/2004
15/09/2004

FIGURE 4

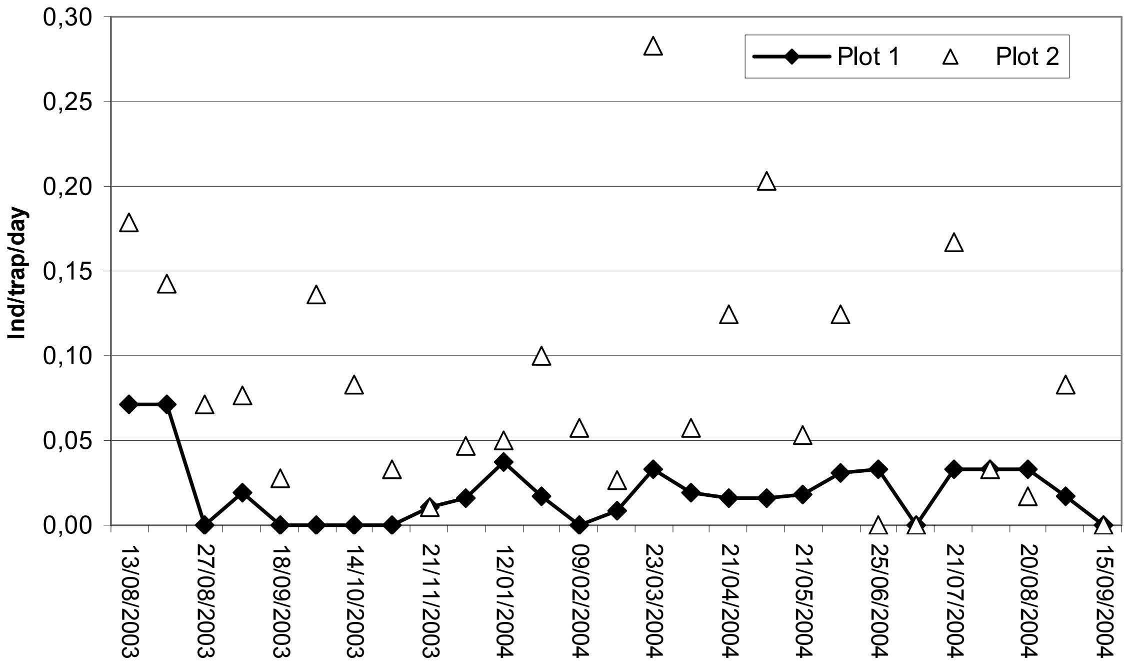

Plot 1
Plot 2
Ind/trap/day
0,30
0,25
0,20
0,15
0,10
0,05
0,00
13/08/2003
27/08/2003
18/09/2003
14/10/2003
21/11/2003
12/01/2004
09/02/2004
23/03/2004
21/04/2004
21/05/2004
25/06/2004
21/07/2004
20/08/2004
15/09/2004

FIGURE 5

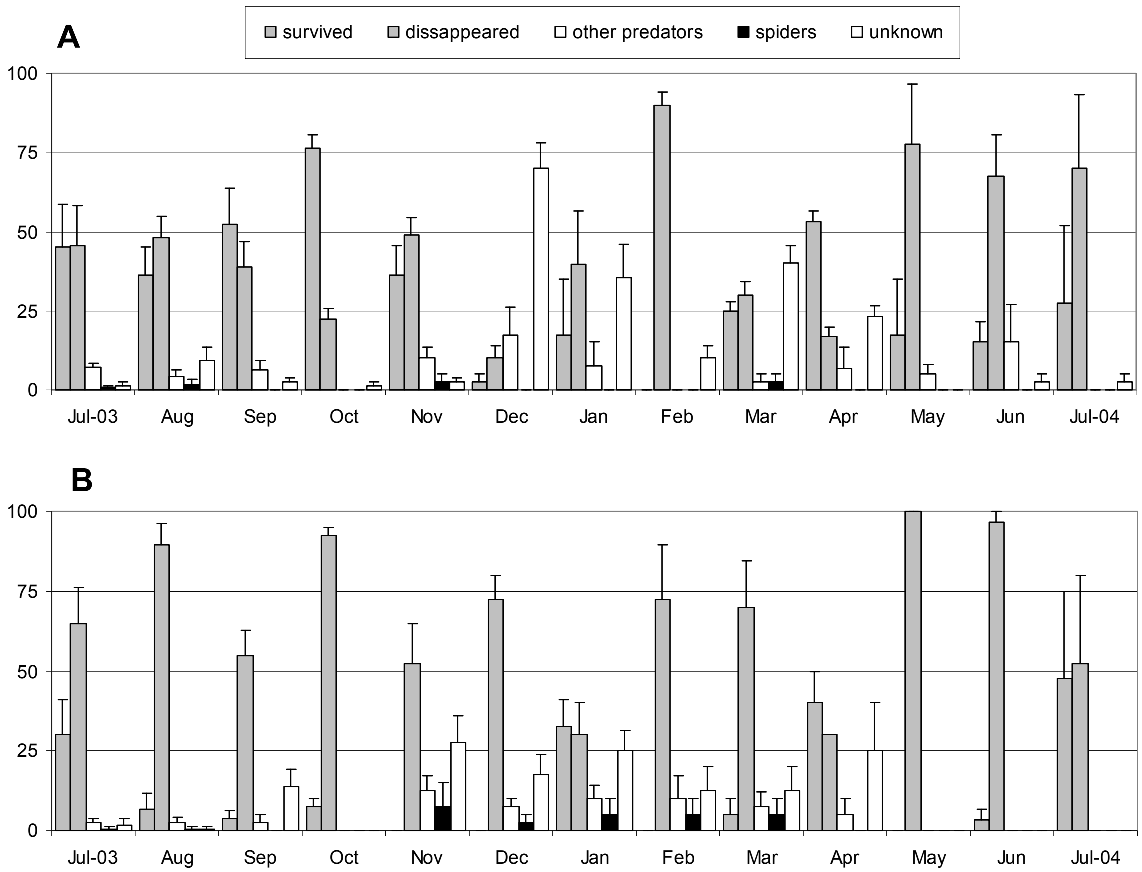
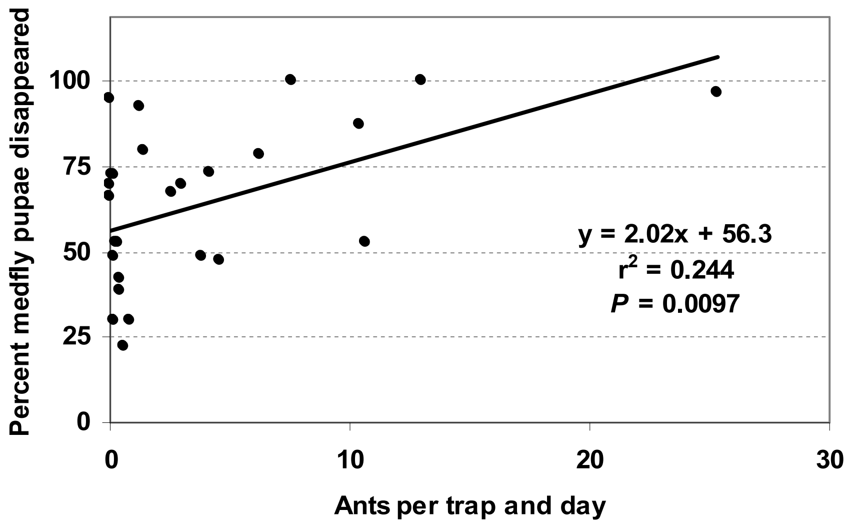

A
survived
dissappeared
other predators
spiders
unknown
100
75
50
25
0
Jul-03
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul-04
B
100
75
50
25
0
Jul-03
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul-04

FIGURE 6


Percent medfly pupae disappeared
100
75
50
25
0
0
10
20
30
Ants per trap and day
y = 2.02x + 56.3
r2 = 0.244
P = 0.0097